FEBS Letters 583 (2009) 927–932

FEBS Letters

journal homepage: www.FEBSLetters.org

Robustness and topology of the yeast cell cycle Boolean network

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ARTICLE INFO

Article history:
Received 28 November 2008
Revised 2 February 2009
Accepted 3 February 2009
Available online 10 February 2009

Edited by Gianni Cesareni

Keywords:
Yeast cell cycle network
Robustness
Network topology
Boolean network

ABSTRACT

Yeast cell cycle Boolean network was used as a case study of robustness to protein noise. Robustness was interpreted as involving stability of G1 steady state and sequence of gene expression from cell cycle START to stationary G1. A robustness measure to evaluate robustness strength of a network was proposed. Robust putative networks corresponding to the same steady state and sequence of gene expression of wild-type network were sampled. Architecture of wild-type yeast cell cycle network can be revealed by average topology profile of sampled robust putative networks.

1. Introduction

Robustness against external and internal perturbations is a fundamental feature that had been widely observed in many biological systems, e.g. cell cycle, segment polarity in Drosophila, chemotaxis in bacteria, cancers, immune system, etc. [1–7]. The perturbations can be gene mutation, interaction deletion/addition, transcription/translation noise, external environmental stimuli and so on. Robustness enables cell to maintain its physiological behaviors against perturbations, i.e. keeping up its normal functions. As pointed out by Kitano, robustness of a biological system should manifest itself in keeping up its normal functionalities [2]. In order to maintain functionalities, biological system is probable to change its steady state in response to external stimuli, and the intermediate gene expressions are responsible for specific functions. For example, minimal model of eukaryotic cell cycle control network should be composed of cyclins, cyclin-dependent kinase (CDK), regulatory proteins of cyclin-CDK complexes, transcription factor of cell cycle components. Cell cycle start is triggered by a cell mass related external stimulus. After start of cell cycle, the gene expression is dynamically changed which corresponds to G1, S, G2, and M phases of cell cycle events and then back to stationary G1 (steady state). In order to keep success of cell cycle, gene expression of cell must proceed in correct order of Gl → S → G2 → M → stationary G1. Hence, not only the steady state but also the sequence of dynamic gene expression during cell cycle process should be robustly designed [14]. Robustness study of biological networks was initially focused on stability of steady state [8–11]. In stead of only considering steady state, Li et al. studied budding yeast cell cycle network, they claimed that robustness of yeast cell cycle network involved both a global steady state and a state attractive dynamic gene expression trajectory [14,15]. This ensures yeast cell to proceed in correct order of cell cycle events, G1, S, G2, M phases and then back to stationary G1 phase.

Robustness and fragility are one thing in opposite sides. Robustness analysis helps one to identify essential interactions to a robust biological system, and these essential interactions could be potential drug targets [16]. It is generally accepted that network topology determines robustness of a biological system. Dynamically robust network motifs were discovered to be more abundant in real biological networks [17,18]. However, the design principle of a real robust network is unclear yet, only some rules were proposed to design an artificial robust network [19]. Robustness analysis of biological networks in structural perspective enables one to search drug target candidates and disease therapy [3,4,16]. Unfortunately, the information of network structure is usually lacked or incomplete. Reverse engineering approach of systems biology is to infer network structure from time series gene expression profiles and some additional required knowledge [20–22]. Nonetheless, network structures are highly degenerate to gene expression profile. Insufficient time points and noisiness of gene expression data make the difficulties to reconstruct network [23]. Without the network structure, it is impossible to analyze dynamical robustness of a

Abbreviations: YCC, yeast cell cycle; SIT, signal input trajectory; MFPL, maximal flux pipe line

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0014-5793/$34.00
doi:10.1016/j.febslet.2009.02.010

biological system. In contrary to analyze robustness from network structure, plausibility of biochemical network models had been evaluated by robustness analysis [25,26]. Biological function constrains diverse network structures of a biological system [24]. But constraints on network topology of stability of biological function, i.e. robustness, is unclear yet. One is interesting to ask how much information of network topology can one get from robustness? Is it possible to extract network topology by robustness analysis?

In this paper, we will use wild-type yeast cell cycle (YCC) network by Li et al. as a case study [14]. The dynamical robustness of YCC network under protein noise attack was investigated by Boolean network model. Robustness was examined in terms of having a dominant maximal flux signal input trajectory (SIT) and a global steady state. Here SIT means the sequence of gene expression from cell cycle START to stationary G1 (steady state). A novel robustness measure of YCC network was proposed. Networks of the same function with YCC network were sampled. With use of this proposed robustness quantity, these sampling networks can be classified into robust and fragile groups. Topology of wild-type YCC network could be extracted from these sampling robust networks. Robustness analysis may be a potential guide line for use of network topology identification.

2. Materials and methods

The wild-type YCC network proposed by Li et al., as shown in Fig. 1, was used to simulate stochastic dynamics with Boolean network [14]. Adjacency matrix W_{ij} of this network was defined as $W_{ij} = 1$ if protein j activates protein i, $W_{ij} = -1$ if protein j inhibits protein i, and $W_{ij} = 0$ if protein j does not interact with protein i. The role of parameters was neglected here. YCC network had been proved to have a permissible range of parameters to work robustly by experiment [12]. Hence, Boolean network is appropriate to study robustness of YCC network [11,13]. We simplify $X_i(t)$ (expression level of protein i at time t) to binary states, the active ($X_i(t) = 1$) and inactive ($X_i(t) = 0$) states. The temporal gene expression level of a specific protein i evolved as the following rules:

$$\begin{aligned} P\left(X_i(t+1) = l \middle| \sum_j W_{ij}X_j(t) > 0\right) &= 1-\rho \\ P\left(X_i(t+1) = 0 \middle| \sum_{ij} W_{ij}X_j(t) < 0\right) &= 1-\rho \\ P\left(X_i(t+1) = X_i(t) \middle| \sum_j W_{ij}X_j(t) = 0\right) &= 1-\rho \end{aligned} \quad (1)$$

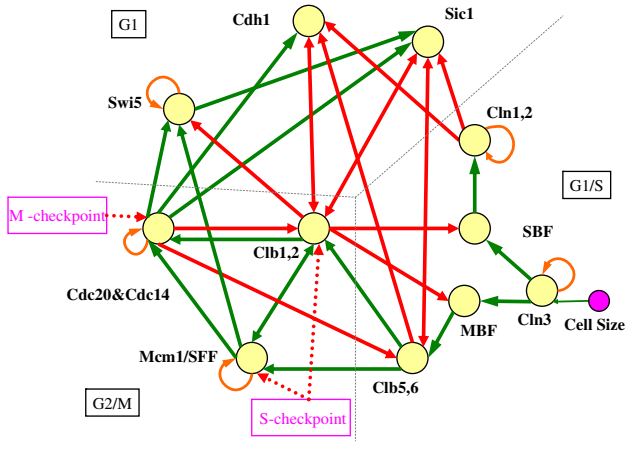

Fig. 1. Model of yeast cell cycle (YCC) network proposed by Li et al. The red and green arrows indicate inhibitory and activated interaction, respectively.

the state of protein i becomes active or inactive at time $t+1$ depending on it was up regulated ($\sum_j W_{ij}X_j(t) > 0$) or down regulated ($\sum_j W_{ij}X_j(t) < 0$) at time t. Though sources of protein noise are diverse, proteins with expression level higher than a threshold were observed to have constant noise [29]. Hence, we assigned a constant state flipping probability ρ to simulate such protein noise in Eq. (1).

3. Results and discussions

Boolean dynamics of YCC network, as depicted in Fig. 1, in the absence of protein noise was investigated by Li et al., i.e. $\rho = 0$ in Eq. (1). They pointed out that there are seven steady states in this network. Only the steady state of largest basin size is biologically relevant. The sequence of dynamic gene expression from cell cycle START to steady state (SIT), as shown in Table 1, was argued to be qualitatively consistent with gene expression of cell cycle [14].

3.1. YCC network is robustly designed

3.1.1. Robustness involves stability of steady state and SIT

Protein noise is ubiquitous in cellular system, and cell cycle functions robustly in such noisy environment. When cell stops to divide, cell will stay in stationary G1 phase gene expression. As cell cycle was triggered, gene expression follows SIT proceeded in a specific order corresponding to G1, S, G2 and M phase events, and then back to stationary G1 phase expression. Here not only stationary G1 phase but also transient phases G1, S, G2 and M are biological significant. Damage of any stage of gene expression during cell cycle process will cause severe cell cycle defects. Hence, both the steady state and sequence of transient gene expression must be designed robustly. Most robustness study of biological systems focused on stability of steady state, but is seldom to take transient gene expression into account [9,10,14,15,27,28]. Robustness of YCC had been explained by a pseudo-potential energy funneled toward steady state [9,10,15]. Sequence of gene expression during cell cycle process is a deep valley in the pseudo-potential energy landscape to avoid gene expression to deviate from normal expression [15].

Here we used stochastic Boolean network model to investigate robustness of YCC network under protein noise perturbation. We found that the most likely occupied state corresponds to stationary G1 phase (steady state in the absence of noise). SIT overlaps with the maximal flux pipe line (MFPL). MFPL is the path of largest flux among all possible paths flowing into steady state. Being the most favorite occupied state of stationary G1 tells us that gene expression of yeast cell will not deviate from stationary G1 easily before its reentry into cell cycle. Under a given appropriate protein noise strength, the sequence of states on SIT overlap entirely with MFPL. Sequence of gene expression in a cell cycle followed the global maximal flux path to steady state. And also, SIT dominates over all paths. The flux of second largest path differs in two order of magnitude with SIT in protein noise strength $\rho = 0.04502$, as in Fig. 2. Any prominent path from some state to steady state must be a part of SIT. Hence, robustness of YCC under protein noise attack should manifest in having a most favorite occupied stationary G1 state and SIT is the unique prominent path from cell cycle START to stationary G1.

3.1.2. Quantitative robustness measure of YCC network

In the above discussions, it is necessary for a robust YCC network to satisfy two conditions under protein noise attack. The first, steady state is the most favorite occupied state; the second, SIT kept itself to be a part of MFPL when YCC network was perturbed by an appropriate strength of protein noise. Any one of the two conditions was violated, YCC network failed to be robust. Quantita-

Table 1
Signal input trajectory (SIT) of wild-type yeast cell cycle (YCC) network.

Time	Cln3	MBF	SBF	Cln1,2	Cdh1	Swi5	Cdc20&Cdc14	Clb5,6	Sic1Cl	Clb1,2	Mcm1/SFF	Phase
1	1	0	0	0	1	0	0	0	1	0	0	START
2	0	1	1	0	1	0	0	0	1	0	0	G1
3	0	1	1	1	1	0	0	0	1	0	0	G1
4	0	1	1	1	0	0	0	0	0	0	0	G1
5	0	1	1	1	0	0	0	1	0	0	0	S
6	0	1	1	1	0	0	0	1	0	1	1	G2
7	0	0	0	1	0	0	1	1	0	1	1	M
8	0	0	0	0	0	1	1	0	0	1	1	M
9	0	0	0	0	0	1	1	0	1	1	1	M
10	0	0	0	0	0	1	1	0	1	0	1	M
11	0	0	0	0	1	1	1	0	1	0	0	M
12	0	0	0	0	1	1	0	0	1	0	0	G1
13	0	0	0	0	1	0	0	0	1	0	0	Stationary G1

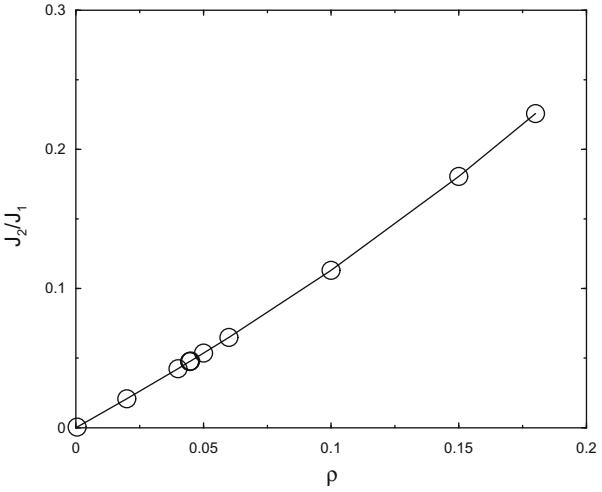

Fig. 2. J_1, J_2 are the maximal and second large flux flowing into a system state on signal input trajectory (SIT). It showed the value of J_2 to J_1 ratio averaged over states on SIT under various strength of noise. J_2/J_1 is smaller than 0.042 when the noise is weaker than critical noise $\rho_c = 0.04502$.

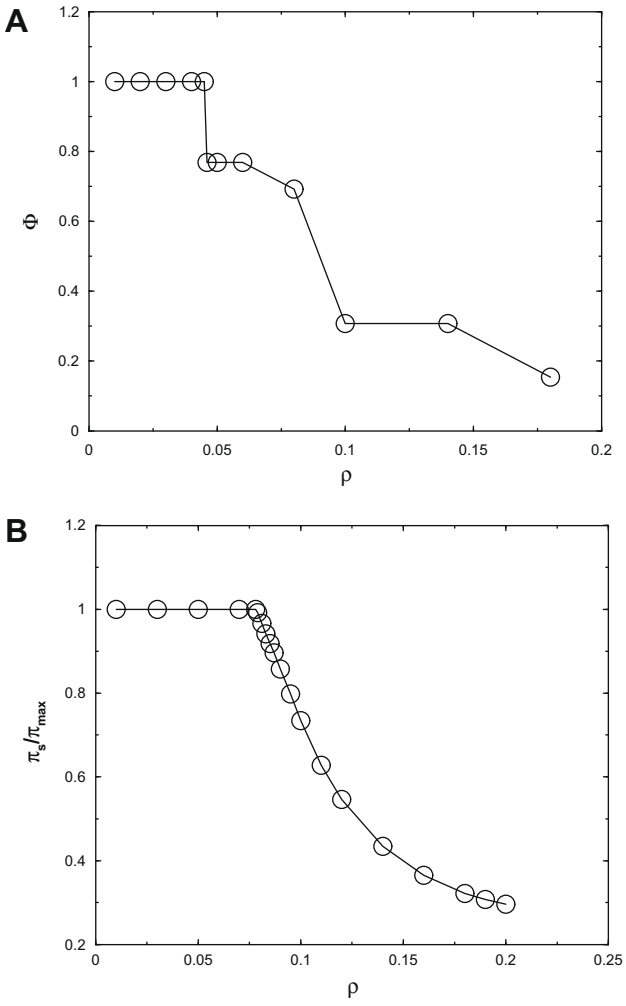

Fig. 3. Robustness measure of wild-type yeast cell cycle (YCC) network. (A) Φ is the fraction of overlapping states on signal input trajectory (SIT) with maximal flux pipe line (MFPL). The robustness measure ρ_c of SIT is defined as the noise strength as Φ becomes smaller than 1. (B) The steady state π_s to maximal occupied state probability π_{max} ratio under various protein noise strength. The robustness measure of steady state ρ_s is defined as the noise strength when steady state is no more the most preferable state.

tive robustness measure of YCC had been proposed before, but they only focused on stability of steady state, the tightness of sequential gene expression during cell cycle was neglected [2,8–11,15]. Here we proposed two novel indices ρ_c and ρ_s to quantitative measure robustness of SIT and steady state of YCC network respectively. ρ_c was defined as the critical strength of noise that SIT began to deviate from MFPL. ρ_s is the critical strength of noise that stationary G1 expression is no longer the most favorite occupied state. In Fig. 3, we see that stability of sequential order of transient gene expression becomes fracture beyond $\rho_c = 0.04502$. As the noise strengthens beyond $\rho_s = 0.0785$, the stationary G1 expression is no longer the state of maximal occupied probability. The system states become randomly distributed.

Usually, the value of ρ_c is smaller than ρ_s. It means that stability strength of sequential order of gene expression during cell cycle is weaker than steady state. Hence, we could only use ρ_c to measure how stable the YCC network is. And also, the value of ρ_c depends on network topology. The larger value of ρ_c, the more stable is the network. Hence, ρ_c could be used as a good quantity to evaluate robustness of a network.

3.2. Robustness and network topology

3.2.1. Wild-type YCC network is relative more robust

In order to understand the interplay between network topology and robustness, we generated networks by adding or deleting interactions from wild-type network. A network is said to be robust if it has the same steady state and SIT with wild-type YCC network, and has nonzero robustness measure ρ_c. We sampled 4000 robust networks by adding or deleting interactions from wild-type YCC network. These generated robust networks are called putative

YCC networks. They are possible candidates of YCC control network in evolutionary perspective. We defined a quantity Δn to be the number of interaction differences between a putative network and wild-type network. Δn could be used to measure structural differences quantitatively. Regulatory interactions could be changed due to point mutation, even a single point mutation occurred in promoter or protein binding domain could affect binding affinity between protein and DNA or proteins greatly. Mutation effect on YCC network can be simulated by structural change. When we sampled robust putative YCC networks, we found that every robust putative YCC network can generate another robust network by single interaction changed. There is a nonzero probability ρ_m for a YCC network survive to be robust after changing of its structure by mutation. If point mutation is neutral, the structural difference quantity Δn can be seen as evolutionary time needed for a putative YCC network to evolve to wild-type network topology. The more structural differences, the more time is needed to evolve from a putative network to wild-type network. Another interesting thing during putative networks sampling process is that many putative networks possess the same SIT with wild-type network in the absence of noise, but they have zero critical noise strength $\rho_c = 0$. These networks could only work in absence of noise, no matter how weak is the noise they become fragile when noise is present.

To investigate the correlation between network structure and robustness, the sampled networks were classified by Δn into groups from $\Delta n = 1$ to 20. As seen in Fig. 4A, robustness measure ρ_c of putative YCC networks is negative correlated with Δn with correlation coefficient −0.89. The wild-type YCC network possesses relative more robust network structure among these putative YCC networks. The more structural similarity of a putative network compared to wild-type network, the more robust is it on average. This indicates that YCC networks tend to evolve to more robust topology against protein noise. It is not surprising that more robust networks are superior to survive and have progeny. In Fig. 4B, the critical noise ρ_c is negatively correlated with ρ_m. It told us that more robust networks are more easily harmed by mutation perturbation. Wild-type YCC network fails to be robust with one interaction deleted except five interactions, and four of five perturbed networks become less stable than wild-type network (see Supplementary Table 1).

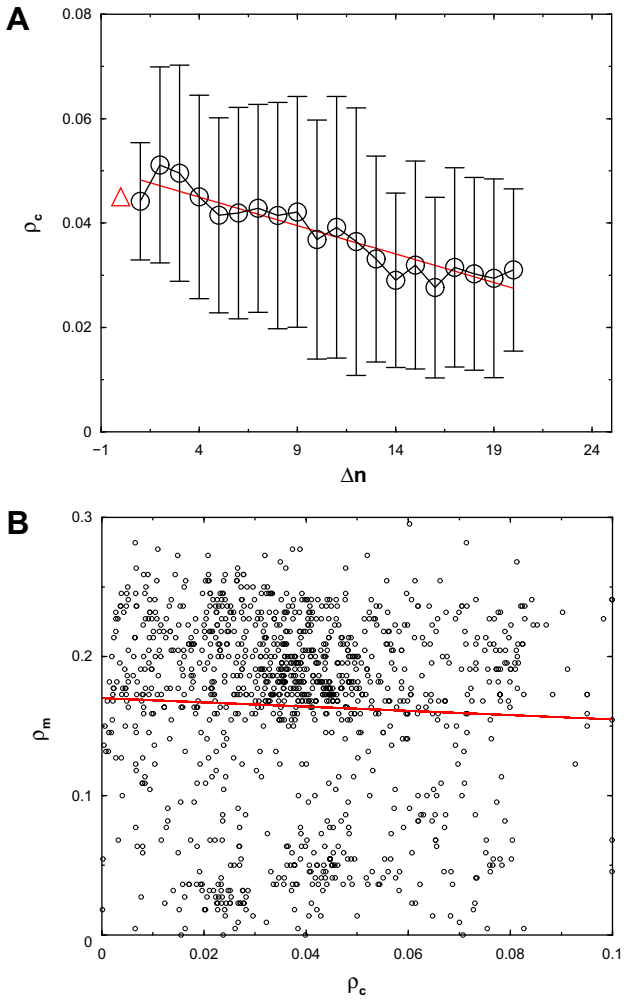

Fig. 4. (A) Triangle shows the critical noise ρ_c of wild-type yeast cell cycle (YCC) network. Circles are ρ_c for sampled putative YCC networks. The critical noise ρ_c is negative correlated with Δn. The correlation coefficient is −0.89 with *P*-value < 0.0001. (B) Figure of ρ_m versus ρ_c. ρ_m and ρ_c are negatively correlated. The slope of regression line is −0.152. A strong noisy robust network is more fragile to mutational perturbation.

3.2.2. In-silico robustness analysis reveals network topology

To understand the interplay between network topology and robustness, we sampled 4000 networks having the same steady state and SIT as wild-type YCC network, we called these functional networks. Functional networks could function in correct order of cell cycle events and then back to stationary G1 in the environment without noise. Despite of this, we found lots of functional networks failed to be robust, i.e. $\rho_c = 0$. Functional networks could be further divided into robust and fragile groups. The fragile functional networks can not be the candidates of natural YCC network. They function with abnormal sequence order of cell cycle events in noisy environment.

We numbered the proteins as 0:Cln3, 1:MBF, 2:SBF, 3:Cln1,2, 4:Cdh1, 5:Swi5, 6:Cdc20&Cdc14, 7:Clb5,6, 8:Sic1, 9:Clb1,2, and 10:Mcm1/SFF. Interaction id $11 * i + j$ was assigned to interaction between protein j and its target protein i. There are 121 links in the network (self interaction included), each with three possible types of interaction, inhibition, activation and no connection.

Topology of robust sampling functional networks were averaged, the appearance probability of each type of interaction between a protein and its interacting target can be calculated. We discovered that average network composed of maximal appearance probability interaction type for each link coincides with wild-type network, as shown in Fig. 5A. From Fig. 5B, the same result was obtained even if sampled networks were limited to $\Delta n \geqslant 10$. Factor of sampling networks with similar structure to wild-type YCC network can be excluded out. We found 14 interactions with appearance probability 1, they are essential to robustness (see Supplementary Table 2). Any one of such interaction was deleted, YCC network became fragile to noise. To distinguish effect of robustness from function constraints on network topology, we focused on fragile group of functional networks. These networks could have normal cell cycle function, but they are harmful to noise perturbation. Average network topology obtained from fragile group of functional networks was shown in Fig. 5C. There is one interaction difference between average fragile network topology and wild-type YCC network. The interaction with id 103 in wild-type YCC network is Cdh1 → Clb1,2, and it becomes Cdh1 → Clb1,2 in average network. If we average fragile group of sampling networks with $\Delta n \geqslant 10$, the obtained average network differs in two interactions with wild-type network. The two differences are interactions with id 82 and 103. In the average network, the two interactions are Cdh1 → Clb1,2 and Swi5 → Clb5,6. While they are Cdh1 → Clb1,2 and Swi5 has no interaction with Clb5,6 in wild-type YCC network. Lau et al. had been reported that function constrains topology of YCC network [24]. One could extract approximate structure of wild-type network from topology constrained functional networks ensemble. The plausible network

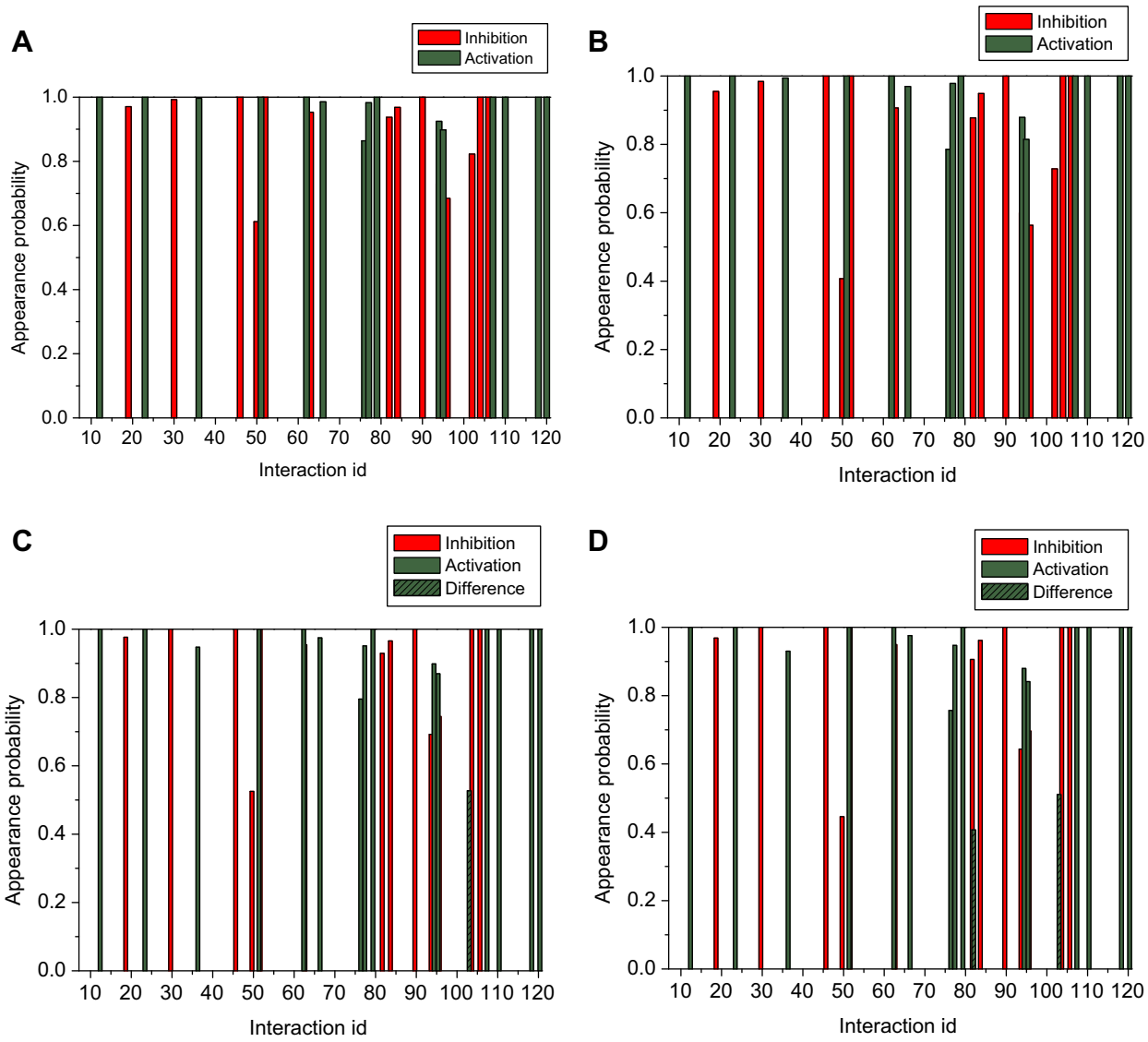

Fig. 5. Activation was represented by green bar, red bar is inhibition. Dominant interactions with no connection type were not shown. (A) Appearance probability of dominant interactions among robust functional networks. (B) Appearance probability of dominant interactions among robust functional networks with $\Delta n \geqslant 10$. (C) Appearance probability of dominant interactions among fragile functional networks. The differences between average dominant interactions and wild-type network interactions were shown as slash bar. (D) Appearance probability of dominant interactions among fragile functional networks with $\Delta n \geqslant 10$.

structure of wild-type network can be obtained by performing robustness analysis on functional networks ensemble.

In the past, robustness had been used as a measure of plausibility of a biological model [25,26]. As our result in yeast cell cycle Boolean network, the real network topology can be revealed by robustness. With appropriate robustness description of the yeast cell cycle Boolean network is offered, i.e. robustness must involve the ability of the yeast cell cycle Boolean network to maintain its normal function under perturbation, robustness could be successfully used as a strategy to unravel yeast cell cycle Boolean network architecture. The approach mentioned above is a computational result on a toy mathematical yeast cell cycle model and more evidences are needed to validate this approach.

4. Conclusions

YCC Boolean network was used as a case study of robustness to noise perturbation. Robustness involves stability of the G1 steady state and sequence of gene expression form cell cycle START to stationary G1. By sampling topology of robust networks, yeast cell cycle network architecture was found to be able reconstructed from average topology profile of sampled robust networks. Cell cycle is a long history evolved and conserved system, it had been adapted to be robust. Robustness can be used as a good strategy to infer or evaluate cell cycle network in other species. If one could identify key regulators of cell cycle, say from clustering time series microarray data, robustness analysis helps to get structure of cell cycle network. For other biological networks emerged with sufficient evolving time, its network architecture may also be revealed by robustness if proper robustness was defined. Robustness could be useless to get network structure for recently emerged biological networks which may have not adapted to be robust.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.02.010.

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